Determination of Unsaturation Ratios for Long Chain Ketones by High Resolution Mass Spectrometry-II

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Introduction: We previously reported on the use of high resolution MS to determine the degree of unsaturation of long chain (C37) alken-2-ones ("alkenones") biosynthesized by a class of marine algae (*Haptophyceae*) (1). Alkenone unsaturation has been shown to be related to algal growth temperature (2). Our previous work demonstrated an MS alkenone ratio analysis for sample amounts comparable to those required for GC-FID. The availability of a set of independently analyzed intercalibration samples and the continued need for a simple, robust and sensitive alkenone ratio measurement technique prompted further investigation of the MS method.

Methods and Instrumentation: Alkenone standards and samples that had been independently analyzed as part of an international intercalibration exercise were analyzed on a VG Autospec MS. For the purposes of response quantification, GC-FID was used to determine the actual concentration of components in each sample. Sample volumes were then adjusted so that the component of lesser concentration was present at about 1ng/μl. Samples were desorbed from a resistively heated platinum wire probe using 20eV direct electron ionization with the MS tuned and acquiring SIR data at 20,000 resolution. A calibration curve based on provided values for the intercalibration exercise standards was used to convert the MS obtained ratios for the samples into haptophyte growth temperature.

Results and Discussion: An investigation of the relationship between raw alkenone unsaturation ratio response and ionization energy revealed that a 10-fold sensitivity increase could be obtained by increasing from 12 to 20 eV. Any changes in raw alkenone ratio that this induces are trivial since an MS ratio to haptophyte growth temperature conversion is required in any case. Acquisition of SIR, as opposed to continuum, data was shown to further increase sensitivity. The net effect of these changes was calculated to be a 50-fold decrease in sample requirement to achieve the same precision as previously reported. Further, the dramatic reduction in amount of material resulted in a significant increase in number of samples that could be analyzed before source cleaning was required.
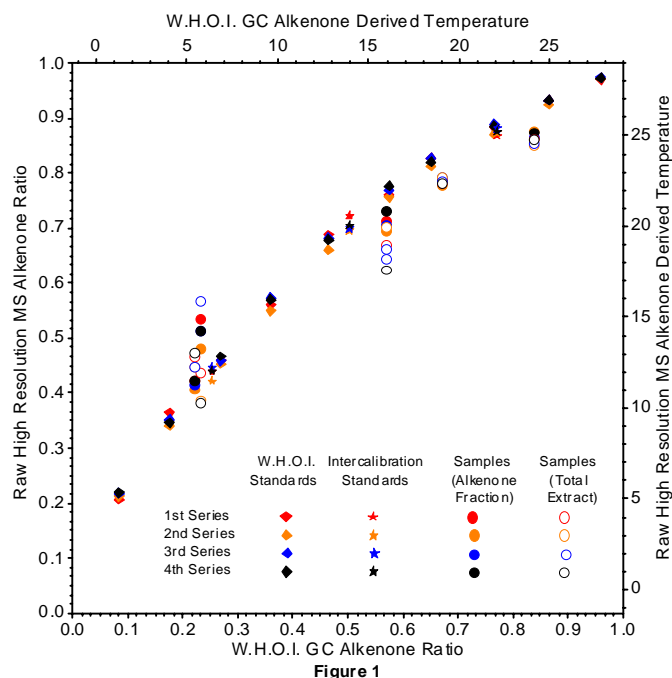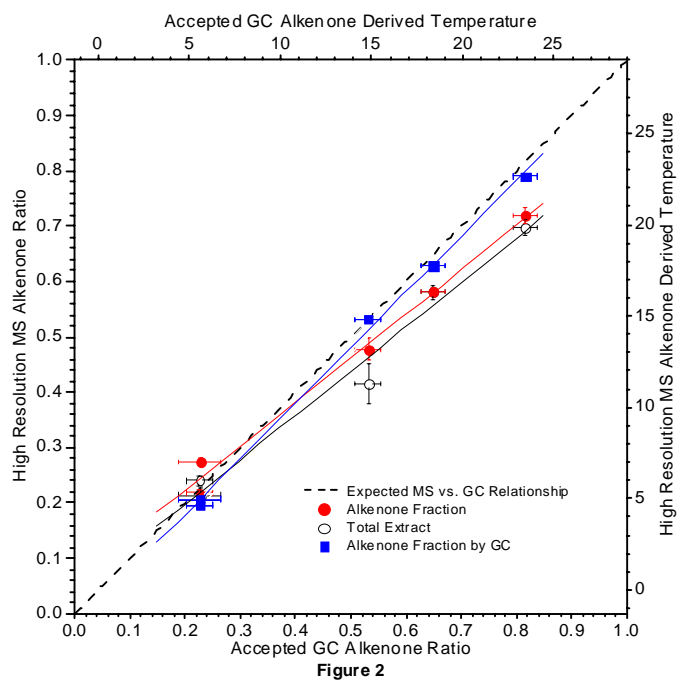

Figure 1

Figure 1 presents the total raw data set plotted as a function of the alkenone ratio determined by GC at W.H.O.I. A suite of three standards and five samples from the intercalibration exercise was analyzed, along a set of W.H.O.I. alkenone standards, in quadruplicate, over the course of several days. Note that both the intercalibration and W.H.O.I. standards fall very tightly along a single smooth curve, indicating that correlation between the GC and MS alkenone ratios is possible for highly purified samples irrespective of their origin.

Figure 2

In figure 2, a haptophyte growth temperature was determined for each replicate sample based on the intercalibration standard curve generated for that run sequence. The average MS precision for the f1's is seen to be +/- 0.3 °C which is significantly better than the precision of the accepted values. The MS values tend towards lower values with increasing temperatures. The average MS value are 1.1 °C lower than the accepted values.

Several possible causes exist for the differences between the MS and accepted values. It is possible that differences in extraction or analytical protocols could result in differences in the final concentrations or ratios of alkenones reported. For the Intercalibration Exercise, each group received raw sediment which was analyzed using that group's standard protocol. For our samples, the MS data shows a 1°C increase from the total extracts to the f1's indicating that some discrimination is occurring during chromatography. Also, the average result for the W.H.O.I. f1's is about 0.6 °C lower than the overall intercalibration accepted values.

Preliminary investigation by two-dimensional GC indicated the presence of coeluting compounds in the f1's which had not been detected by single dimensional GC. These same coeluting materials were not seen when the intercalibration standards were subjected to the same two-dimensional GC analysis. However, the removal of the interfering compounds from the f1's would tend to shift the GC ratios to higher temperature and thus generally further degrade the correlation between the GC and MS methods.

It is also possible that other compounds of the very similar mass may be eluting from the MS probe. However, visual inspection of 25,000 resolution continuum MS data shows only single well formed and resolved peaks within the respective mass windows used to acquire the SIR data.

Conclusions: We have demonstrated that high resolution mass spectrometry in SIR mode holds promise for being a quick and sensitive alternative to GC for the determination of alkenone ratios. However, before this method can be routinely used, it will be necessary to definitively determine the source of the differences between the ultimate temperature results from this method and those from the GC method.

References:
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